TOXICITY OF METHOPRENE TO ALL STAGES OF THE SALT MARSH COPEPOD, *APOCYCLOPS SPARTINUS* (CYCLOPOIDA)

LADDAWAN BIRCHER AND ERNEST RUBER

Biology Department, Northeastern University, Boston, MA 02115

ABSTRACT. The toxicity of methoprene to the salt marsh copepod *Apocyclops spartinus* was evaluated and compared with sensitivity of mosquito larvae. All stages of the life cycle were tested at concentrations ranging from 0.1 to 10.0 ppm. Eggs and the earliest hatched stages, nauplius I–III were most sensitive to methoprene, with little mortality seen in the later stages. Toxic effects were manifested as death, or failure of eggs to hatch, however, no extensions of the life cycle were observed. In general the copepods were resistant at concentrations of methoprene used to control mosquitoes. Early nauplii, however, did show some mortalities to methoprene concentrations near the lower margins of mosquito susceptibility. This might lead to transient decreases in copepod population growth rates, but not necessarily to decreases in their standing populations.

INTRODUCTION

Metamorphosis in insects is partly controlled by the corpora allata. These secrete juvenile hormone, and when this secretion declines the insect undergoes metamorphosis. Juvenile hormone activity has been found in extracts of many invertebrates and plants. One of the earliest synthesized compounds which showed such hormonal activity was farnesol (Williams 1967). Spielman and Williams (1966) reported a 10 ppm dose of ethanolic extracts of farnesoic acid with hydrogen chloride to be effective in blocking the emergence of adult mosquitoes, the most sensitive stage being the late, fourth instar larva. The insect treated with such extracts is unable to complete its metamorphosis and eventually dies.

A number of synthetic insect growth regulators have been evaluated for effectiveness against mosquitoes. Among these, one which has been registered and is in broad use is methoprene (Rathburn 1979, Rathburn et al. 1979, Williams and Palmisano 1981). The toxic effects on non-target species of methoprene and other growth regulators have been the subject of a number of studies (Miura and Takahashi 1973, Steelman et al. 1975, Barber et al. 1978, Case and Washino 1978, Self et al. 1978, Ellgaard et al. 1979, Ali and Lord 1980).

Only Miura and Takahashi (1973) evaluated the effect of methoprene on copepods, an important non-target group of microcrustacea. They reported a 24-h LC_{50} of 4.60 ppm Altosid® (methoprene) for mixed stages of the copepod, *Cyclops* sp. but found no apparent effects on copepods in irrigated fields treated for mosquito control, nor in an experimental pond treated at a rate of 0.1 lb./acre. However, their graph (p. 921) for the pond treatment showed an increase of *Cyclops* densities in the controls which was not observed in the methoprene treated pond. Because of this possible effect on reproduction, and because no one has examined the susceptibility of each life cycle stage of a copepod under laboratory conditions, we performed such a study for the cyclopoid copepod, *Apocyclops spartinus* (Ruber), which is commonly found in habitats of salt marsh mosquito larvae.

MATERIALS AND METHODS

Apocyclops spartinus was described from the salt marshes of New Jersey. The genus occurs in shallow, stagnant, saline waters in many places around the world (Ruber 1968). The present stock was collected from salt marsh pools of the Parker River National Wildlife Refuge in Rowley, Massachusetts. We have been able to maintain this copepod for years in 1-gallon (19 liter) jars held at room temperature with salinity ranging from 10 to 30 parts per thousand. During experiments, they are fed every 3 to 4 days on a hay infusion on which air-borne bacteria have been permitted to grow. For general colony maintenance they are fed only every 7 to 10 days. During experiments the copepods were maintained in artificial seawater at 25 ppt. This water had a hardness equivalent to 4548 mg/liter $CaCO_3$ and its pH varied from 7.8 to 8.1. Stock cultures maintain dissolved oxygen levels of greater than 60% saturation. The copepods were kept in small depression plates of 3 ml capacity during experiments. Oxygen was not at this time determined but we assume that with the large surface area to volume ratio of these depressions, that oxygen was at least as high as in the larger, colony containers.

The female *A. spartinus* carries from 6 to 15 eggs in each of 2 external egg sacs. These hatch into nauplii of which there are 6 stages followed by 5 copepodite stages and finally the adult. All immature stages except eggs were obtained from colony-reared female *Apocyclops*. They were treated with concentrations of technical grade methoprene (Zoecon Corp., Altosid SR-90) ranging from 0.01 to 10 ppm. The compound

was diluted first in acetone, next into distilled water, and finally added to test chambers and natural culture water to achieve the desired final concentration. Controls consisting of the highest acetone concentrations (100 times that of the methoprene) were run with each test. Copepods were placed in small numbers (1–2) in plastic depression plates of 3 ml capacity and held at room temperature (22–24°C) to determine 48-h LC_{50} values. Three sets of replicates were run for each treatment, with the smallest number in a set being 5, the largest, 21, and most ranging from 8 to 15.

Eggs were treated while attached to the female copepod. Mobility of the females was reduced by chilling them on a microscope cold stage, their eggs were counted, and the females were permitted to rewarm to room temperature. They tolerated this treatment without mortality. Adults were removed from test chambers after the eggs hatched. Egg LC_{50} was determined at 72-h, the time interval within which eggs normally hatch.

A separate series of experiments was designed to determine whether there was some particular stage of the lifecycle at which copepods were more likely to die. Eggs were treated with methoprene at concentrations of 0.1–2.0 ppm. Newly hatched nauplii were observed and regularly censused at different stages until death or maturation (7–14 days) occurred.

Aedes sollicitans (Walker) larvae were collected as needed from a salt marsh in Kittery, Maine and kept in the same medium as the copepods. Other *Aedes* sp. larvae were collected from freshwater flood plains of the Sudbury River in Sudbury, Massachusetts. These were kept in their original habitat water for which we have no chemical data.

Mosquito larvae were sorted to instar in groups of 50 and treated in screened, flat aluminum pans which held a liter of water to a depth of 4 cm. They were then treated with methoprene over the same range of concentrations as were the copepods. Small amounts of powdered dog food were added every three days. Observation continued until adults eclosed or immatures died. *Aedes sollicitans* were collected and tested during the summer, hence these were kept at room temperature (22–25°C) during tests. The freshwater *Aedes* were obtained and tested during spring. In order to simulate field conditions they were placed in a cold room at 13°C during nighttime and in a warmer room (22–24°C) during daytime.

RESULTS

To treat large enough numbers for statistical comparison, 48-h mortality tests were grouped into 5 stages; early (N_1 to N_3) and late (N_4 to N_5) nauplii, early (C_1 to C_3) and late (C_4 to C_5) copepodids, and adults. Egg LC_{50}, defined as failure to hatch at 72-h, was between 1.0 and 2.0 ppm. Early nauplii were most sensitive, with a 48-h LC_{50}, adjusted for control mortality of 0.8 ppm; late nauplii had an LC_{50} of 2.0 ppm (Fig. 1). Later stages were progressively more resistant with early copepodids showing a 48-h LC_{50} of approximately 5 ppm and late copepodid and adults having LC_{50} values of greater than 10 ppm, the limit of our experimental concentrations (Fig. 2).

Treated and control eggs were also held until maturation or death, with survival to various stages (and ages at which these were attained) being recorded (Table 1). The main objective was to determine whether mortalities tended to

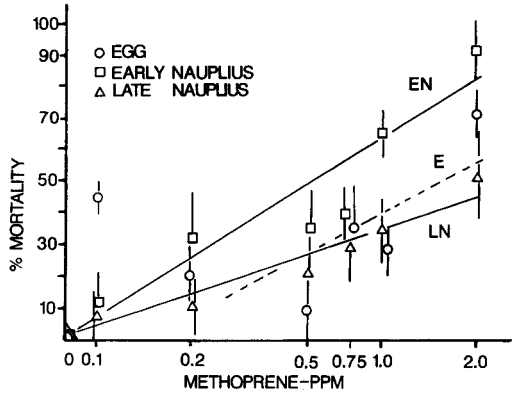

Fig. 1. Effect of methoprene on mortality of early life cycle stages of *Apocyclops spartinus*. Mortalities (48-h) as excess above controls ± SE. Treated eggs respond erratically.

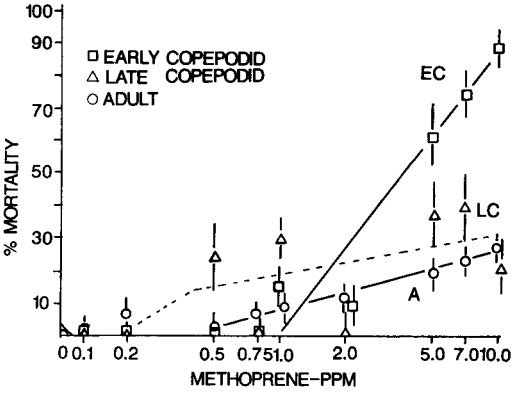

Fig. 2. Effects of methoprene on mortality of later life cycle stages of *Apocyclops spartinus*. Mortalities (48-h) as excess above controls ± SE. Late copepodites respond most erratically, but together with adults are clearly the least sensitive stages of the life cycle.

Table 1. Mortality (%) between different developmental stages of *Apocyclops spartinus* after eggs are treated with methoprene.

	Stage and (day of stage)					
	Egg (0)	Early nauplius (3)	Late nauplius (7)	Copepodid (8)	Adult (10)	Total
Control		10 (35.7)[a]	0 (0)	2 (7.1)	16 (57.1)	28
All methoprene		40 (72.8)	7 (12.7)	3 (5.5)	5 (9.1)	55

[a] ( ) = % mortality/total % mortality.

cluster at any particular stage of metamorphosis. The most consistent effect was that the greatest mortality occurred between the egg and the earliest hatched stage, nauplius 1. This did not change with methoprene concentrations, but did differ from controls. Mean mortality for all methoprene treated eggs (0.1 to 2.0 ppm) was 40% during this interval, or 72.8% of all mortality observed through maturation. Control eggs had a 10% mortality during the same interval, this being equivalent to 35.7% of all mortality through maturation. Controls had a much higher proportion of their total mortality between the copepodid and adult stages (57.1%) than did methoprene treated eggs (9.1%).

In the tests of mosquito larvae, we obtained mortalities of 64% at 0.01 ppm for salt marsh *Aedes sollicitans* treated as first and second instar larvae and 96% mortalities at 0.05 ppm for the freshwater *Aedes* treated as third instar larvae. Actual mortalities of *Ae. sollicitans* were probably higher but were obscured by excessive control mortalities.

DISCUSSION

The stages of the life cycle of *Apocyclops spartinus* most sensitive to methoprene are the eggs and the early nauplii; later stages were progressively more resistant (Fig. 1 and 2). This is contrary to what is found in mosquitoes, where late instar larvae are more susceptible than earlier ones. We found eggs to be somewhat more resistant than early nauplii but this could be a function of the age at which the eggs were treated. Rates of failure of egg hatching were very inconsistent at lower exposure concentrations which suggests that other factors were involved. The importance of timing on mortality in egg applications has been demonstrated in insects (Riddiford 1970). When we followed treated eggs through time to death or maturation, the phase of egg hatching to the first nauplius was consistently the most susceptible interval. No extension of the times of metamorphosis was observed in the copepods. Effects were either direct mortality of copepods or failure of eggs to hatch.

It is difficult to translate field treatments, usually given in lb/acre without reference to water depth, to a laboratory treatment in μg/ml or ppm. To do so, we have, based on our own field observations, assumed average depths of 1 foot (30.5 cm) in treated waters. Based on this assumption various applications ranging from 0.025 lb/acre in fresh waters up to 0.1 lb/acre in salt marsh (Zoecon Corporation data) which are reported to be 90–100% effective in mosquito control would equal, respectively, 0.01 and 0.04 ppm. If water depths were only 6 inches, not unlikely in some cases, the treatment concentrations might be as high as 0.02 to 0.08 ppm. This, of course, ignores questions of formulation which can considerably reduce the release rate.

Several aspects of our study relate to this range of concentrations. First, our own freshwater *Aedes* results correspond reasonably well with the previous citations in that we obtained 96% mortality when third instar larvae were exposed to 0.05 ppm methoprene. With third instar *Aedes sollicitans* (from the salt marsh), we obtained only 64% mortality with 0.02 ppm of methoprene. One problem in the latter case was high control mortality (up to 30%) which reduced our control adjusted test mortality. This aspect of the study was not extended because it was, in our work, secondary to the assessment of copepod susceptibility.

Second, all stages of *Apocyclops spartinus* are far less susceptible to methoprene than are mosquito larvae. We are least confident of this for treated eggs which yielded erratic results, perhaps related to age of the eggs at application. Field concentrations below 0.1 ppm should not cause *Apocyclops* declines.

Third, we considered Miura and Takahashi's (1973) reports on *Cyclops* sp. in a test pond. Their test LC_{50} of 4.60 ppm for "mixed stages" is comparable with the significant effect concentrations which we found for the later stages of *Apocyclops*. As mentioned earlier there was an apparent *Cyclops* increase in their control data which was not mirrored in their treatment data. Their site was a freshwater pond treated at 0.1 lb/acre, which would translate into 0.04–0.01 ppm for water from 1 to 4 feet deep. Miura and

Takahashi reported no decline due to methoprene but they did not address what we interpret as a "missing" population increase in their treatment pond as compared with the control. Given the large variations often seen in field results, and the low susceptibility of the tested copepods we are not able to draw firm conclusions on this point. Early copepod stages are more susceptible (Fig. 1), and a mortality in early nauplii could have resulted in the observed absence of a population surge without necessarily reducing the overall copepod population.

Where methoprene concentrations exceed 0.1 ppm, a concentration beyond that usually needed for control of mosquito larvae, transient effects on early copepod stages might be observed. As regards cyclopoid copepods, methoprene appears to be a relatively safe form of mosquito control, nonetheless, applied doses should be kept to the minimum needed since damage to early stages of these food-chain organisms remains a possibility.

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